69 Rapid induction of high level azithromycin resistance in clinical CF isolates

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Objectives: The use of chronic azithromycin treatment has been linked with increased macrolide resistance amongst respiratory pathogens. The aim of this study was to determine if *in vitro* exposure to azithromycin induces resistance among *Streptococcus* and *Rothia* isolates from CF sputum. We also determined the stability of any resistance induced.
Methods: Development of resistance after serial exposure was investigated by passaging *Streptococcus* (n = 6) and *Rothia* (n = 6) isolates in sub-MIC concentrations of azithromycin for 12 passages, with MICs determined after every passage. The susceptibility of these isolates to a range of antibiotics was also determined before and after serial azithromycin exposure.
Results: High level resistance (MIC >256 μg/ml) developed within 8 passages for 3/6 *Streptococcus* isolates and within 4 passages for 3/6 *Rothia* isolates. Resistance remained stable for these isolates following subculture in drug-free broth. The MIC of the remaining *Streptococcus* and *Rothia* isolates also increased but not to levels above the resistance breakpoints. Furthermore, 2/6 *Rothia* isolates developed resistance to clindamycin following serial exposure to azithromycin.

Table: MIC ranges following azithromycin exposure

	MIC range (μg/ml)			
	Passage 0	Passage 4	Passage 8	Passage 12
Streptococcus	0.064–6	1.5–>256	2–>256	4–>256
Rothia	0.023–0.125	0.064–>256	1–>256	1.5–>256

Conclusion: Exposure of clinical *Streptococcus* and *Rothia* isolates to azithromycin can rapidly induce high level resistance, which appears to be stable.
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70 Expression of RND-type efflux pumps as a mechanism of antibiotic resistance in clinical *Prevotella* isolates

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Objectives: We have previously shown that CF associated *Prevotella* spp. are resistant to antibiotics from multiple classes. It has also been demonstrated that *Prevotella* possess either one or two species-specific RND-type efflux pumps. The aims of this study where to determine if (1) efflux pumps are expressed by *Prevotella* isolates and (2) expression of these pumps contributes to antibiotic resistance.
Methods: Transcription levels of the species-specific efflux pumps were analysed in 31 clinical *Prevotella* isolates and *Prevotella* melaninogenica ATCC 25845 by quantitative real time PCR (qRT-PCR). Minimum inhibitory concentrations (MICs) of ceftazidime (n = 12 isolates), co-amoxiclav (n = 16 isolates) and tetracycline (n = 22 isolates) were then determined by Etest® in the presence or absence of the efflux pump inhibitor (EPI), Phe-Arg β-napthylamide 2HCl. The Wilcoxon Signed Rank test was used to compare MICs before and after inhibition of efflux pumps.
Results: Twenty-six of 31 (84%) *Prevotella* isolates and the type strain expressed the expected RND-type efflux pumps. For the remaining 5 isolates, either no expression was detected (n = 3) or 1/2 expected pumps were expressed (n = 2). A small but statistically significant decrease in MICs for co-amoxiclav ($P = 0.011$, Wilcoxon Signed Rank) and tetracycline ($P < 0.001$) was detected in the presence of the EPI.
Conclusion: Most *Prevotella* isolates express RND-type efflux pumps, which may contribute to co-amoxiclav and tetracycline resistance.
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71 Extended-spectrum β-lactamase (ESβL) production: a mechanism of resistance to ceftazidime in *Prevotella* species isolated from patients with CF

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Objectives: ESβL producing bacteria inactivate ceftazidime, which is used in the treatment of CF lung infection. Ceftazidime resistance is common among CF *Prevotella* isolates. The aims of this study were to determine and compare (1) *in vitro* antimicrobial susceptibility to ceftazidime and (2) ESβL production by *Prevotella* isolates from a range of sources.
Methods: Isolates of *Prevotella* (CF, n = 34; non-CF infections, n = 25; healthy control, n = 17) were tested for susceptibility to ceftazidime by Etest® and for ESβL production using the combined disc method. A chi-square test was used to determine if there was an association between ESβL production and *Prevotella* group. Isolates were split into ESβL positive/negative and MICs compared using the Mann-Whitney test.
Results: Ceftazidime (CF and non-CF, MIC_{90} >256 μg/ml; healthy controls, MIC_{90} 128 μg/ml) resistance was similar between groups. Twenty-five of 34 (77%) CF, 15/25 (60%) non-CF and 11/17 (65%) healthy control isolates were ESβL positive. No association between ESβL production and *Prevotella* group was detected ($\chi^2 = 1.252$, $P = 0.571$). ESβL positive isolates had greater MICs against ceftazidime ($P < 0.001$, Mann-Whitney test) compared to ESβL negative isolates.
Conclusion: ESβL production was common amongst *Prevotella* and was associated with reduced susceptibility to ceftazidime. CF *Prevotella* producing ESβLs may potentially contribute to treatment failure of CF pulmonary infection with ceftazidime.
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72 Inhalatory antibiotic therapy in cystic fibrosis and emergence of colistin resistant Gram-negative non-fermenting bacteria: a new problem in pulmonary infection treatment?

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Objectives: The pulmonary infection in Cystic Fibrosis (CF) patients is characterised by different Gram-negative non fermenting (GNnF) bacteria equipped with intrinsic resistances to antibiotics that make more difficult to treat this infection. Colistin has emerged as a relevant therapeutic option for the treatment of GNnF. Aerosol therapy has become increasingly important in the treatment of CF lung disease. Nevertheless some studies report the emergence of new colistin-resistant (COL-R) pathogens in CF. The aim of this study is to analyse the emergence of COL-R GNnF bacteria in CF patients attending an Italian CF center.
Methods: During 2010–2012 were analysed 1865 strains of GNnF isolated from Genoa CF patients.
The susceptibility to antibiotics was tested by Disk-diffusion and confirmed by E-test. The results were also correlated with clinical data of CF patients.
Results: The number of GNnF is increased from 30 to 58. The main of GNnF recoverd are: *A. xylosoxidans, S. maltophilia* and *P. aeruginosa*. All GNnF strains colistin resistant are multi-drug resistant, except some isolates of *S. maltophilia.* The clinical data showed that the 65% of patients colonised by GNnF were treated with cycles of nebulised colistin and alternated with nebulised tobramycin.
Conclusions: The emergence of COL-R GNnF strains in CF patients treated with nebulised colistin suggest that this therapeutic option can select COL-R strains and can favour the arising of other resistance to antibiotics. Then the isolation of these GNnF bacteria present a real challenge to diagnostic laboratories, as they are difficult to identify and often misidentified as belonging to the *B. cepacia* complex.